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Review

Bacterial phospholipase A: structure and function of an integral membrane phospholipase

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Abstract

Within the large family of lipolytic enzymes, phospholipases constitute a very diverse subgroup with physiological functions such as digestion and signal transduction. Most phospholipases may associate with membranes at the lipid-water interface. However, in many Gram-negative bacteria, a phospholipase is present which is located integrally in the bacterial outer membrane. This phospholipase (outer membrane phospholipase A or OMPLA) is involved in transport across the bacterial outer membrane and has been implicated in bacterial virulence. OMPLA is calcium dependent and its activity is strictly regulated by reversible dimerisation. Recently the crystal structure of this integral membrane enzyme has been elucidated. In this review, we summarise the implications of these structural data for the understanding of the function and regulation of OMPLA, and discuss a mechanism for phospholipase dependent colicin release in *Escherichia coli*. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Phospholipase; Membrane protein; Dimerisation; Colicin secretion; Bacterial pathogenesis

1. Introduction*1.1. Phospholipases*

Lipolytic enzymes form a large family of enzymes which catalyse the hydrolysis of lipids and phospholipids. Phospholipases constitute a very diverse subgroup of enzymes belonging to this family, comprising enzymes with phosphodiesterase as well as acyl hydrolase activity. Of the acyl hydrolases, the 14 kDa disulphide bond rich pancreatic and snake ven-

om phospholipases have been studied in most detail during the past 40 years. More recently, it was found that this group of enzymes, however, is much larger and has a much broader cellular distribution than originally expected [1]. Moreover, other soluble phospholipases, not homologous to the disulphide bond rich phospholipases, have been discovered. Amongst them, the mammalian 85 kDa cytosolic phospholipase [2] has been implicated in inflammatory responses. Yet another member, which has neither sequence homology to the disulphide bond rich phospholipases nor to the cytosolic phospholipases, is the outer membrane phospholipase A from *Escherichia coli*. It is an integral membrane protein located in the outer membrane of many Gram-negative bacteria. Here we review the current biochemical and structural knowledge of this enzyme, outer membrane phospholipase A (OMPLA).

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1.2. History and enzyme characteristics

In 1971 Doi et al. [3] reported a membrane-associated phospholipase A and lysophospholipase activity in *E. coli*. In the same year Scandella and Kornberg [4] isolated and purified a membrane bound phospholipase A₁ from *E. coli*. Some years later, these different activities were attributed to one enzyme located integrally in the outer membrane [5,6]. This 31 kDa enzyme, outer membrane phospholipase A (or detergent-resistant phospholipase, *pldA* protein) appeared to contain 269 amino acids residues preceded by a 20 amino acid signal sequence which targets the protein across the inner membrane [7]. The enzyme is strictly calcium dependent [6] and displays a broad substrate specificity. Besides phospholipase A₁ and A₂ activity the enzyme also harbours lysophospholipase A₁ and A₂ activity, and mono- and diacylglyceride lipase activity. The minimal substrate requirements of the enzyme are a more or less polar head group esterified to an acyl chain of at least 14 carbon atoms [8].

1.3. Activation

OMPLA activity and phospholipid turnover are triggered by such diverse events as phage-induced lysis [9], temperature shift [10], spheroplast formation [11], EDTA treatment [12], polymixin B exposure [13], heat shock [14] and colicin release [15–17]. Furthermore, increased levels of phospholipase A activity have been observed in *E. coli* variants which display decreased stability of the cell envelope (*B. fad* cells [18] and *envC* mutations [19]). All these diverse conditions and triggers have in common that the outer membrane integrity is perturbed, indicating that the activity of OMPLA is correlated with loss of envelope integrity.

1.4. Physiological function

The *pldA* gene is widespread among Gram-nega-

tive bacteria (Fig. 1), indicative of an important function of the gene product. The role of OMPLA has been most thoroughly studied in *E. coli*, where it participates in the secretion of bacteriocins. Bacteriocin release is triggered by a lysis protein (bacteriocin release protein or BRP), followed by a phospholipase dependent accumulation of lysophospholipids and free fatty acids in the outer membrane. The reaction products enhance the permeability of the outer membrane, which allows the semispecific secretion of bacteriocins. [15,17,20,21]. In *pldA*[−] strains, bacteriocin secretion is perturbed and the bacteriocins are accumulated in the cytoplasm [17].

In pathogenic bacteria, phospholipases have been implicated as virulence factors. Grant et al. [22] demonstrated that the *pldA* product is a major haemolytic factor in *Campylobacter coli* [22]. Bukholm et al. [23] identified a *Helicobacter pylori* variant with a high lysophospholipid content in its outer membrane. This variant showed an increased invasive capacity, and an increased VacA and urease release. From these results it has been suggested that release of VacA and urease could be mechanistically similar to bacteriocin release in *E. coli* and that phospholipase A activation is responsible for the increased lysophospholipid levels [23].

The constitutive expression of OMPLA, even in *E. coli* strains that do not possess bacteriocin encoding plasmids, suggests that there must be additional physiological roles for the phospholipase. One speculative function of OMPLA is related to organic solvent tolerance in bacteria. Pedrotta and Witholt [24] postulated that outer membrane perturbations caused by organic solvents would activate OMPLA, resulting in an increased hydrolysis of outer membrane phospholipids and increased release of fatty acids. The fatty acids are a substrate for the periplasmic *cis-trans* isomerase (CTI), an enzyme that isomerises *cis* fatty acids to the *trans* conformation. Phospholipids containing *trans* fatty acids decrease the membrane fluidity and permeability. Such a decrease is part of the solvent tolerance of bacteria.

Fig. 1. Sequence alignment of outer membrane phospholipases produced by Clustal W [42] and Alscript [43]. Residue numbering and secondary structure elements are according to *E. coli* OMPLA. Residues boxed in grey and black are highly and strictly conserved residues, respectively. The catalytic triad is indicated by the black triangles. In the *H. pylori* sequences a long insertion indicated by a box is omitted from the alignment.

10

Residue numbers E.coli

Residue numbers E.coli

Residue numbers E.coli

Residue numbers E.col

Residue numbers E.col

<i>E.coli</i>	Y	N	W	N	T	G	Y	G	G	A	A	E	L	L	G	L	S	S	P	I	T	K	H	V	L	R	L	Y	T	T	O	V	V	S	G	G	Y	G	G	M	M	S	L	L	L	D	Y	N	F	N	Q	T	R	V	G	V	G	V	M	L	N	D	L	F	F			
<i>S.typhimurium</i>	Y	N	W	N	T	G	Y	G	G	A	A	E	L	L	G	L	S	S	P	I	T	K	H	V	L	R	L	Y	T	T	O	V	V	S	G	G	Y	G	G	M	M	S	L	L	L	D	Y	N	F	N	Q	T	R	V	G	V	G	V	M	L	N	D	L	F	F			
<i>S.typhi</i>	Y	N	W	N	T	G	Y	G	G	A	A	E	L	L	G	L	S	S	P	I	T	K	H	V	L	R	L	Y	T	T	O	V	V	S	G	G	Y	G	G	M	M	S	L	L	L	D	Y	N	F	N	Q	T	R	V	G	V	G	V	M	L	N	D	L	F	F			
<i>K.pneumonia</i>	Y	N	W	N	T	G	Y	G	G	A	A	E	L	L	G	L	S	S	P	I	T	K	H	V	L	R	L	Y	T	T	O	V	V	S	G	G	Y	G	G	M	M	S	L	L	L	D	Y	N	F	N	Q	T	R	V	G	V	G	V	M	L	N	D	L	F	F			
<i>P.vulgaris</i>	Y	N	W	N	T	G	Y	G	G	A	A	E	L	L	G	L	S	S	P	I	T	K	H	V	L	R	L	Y	T	T	O	V	V	S	G	G	Y	G	G	M	M	S	L	L	L	D	Y	N	F	N	Q	T	R	V	G	V	G	V	M	L	N	D	L	F	F			
<i>Y.pesitis</i>	Y	N	W	N	T	G	Y	G	G	A	A	E	L	L	G	L	S	S	P	I	T	K	H	V	L	R	L	Y	T	T	O	V	V	S	G	G	Y	G	G	M	M	S	L	L	L	D	Y	N	F	N	Q	T	R	V	G	V	G	V	M	L	N	D	L	F	F			
<i>Y.pseudotuberculosis</i>	Y	N	W	N	T	G	Y	G	G	A	A	E	L	L	G	L	S	S	P	I	T	K	H	V	L	R	L	Y	T	T	O	V	V	S	G	G	Y	G	G	M	M	S	L	L	L	D	Y	N	F	N	Q	T	R	V	G	V	G	V	M	L	N	D	L	F	F			
<i>E.agglomerans</i>	Y	N	W	N	T	G	Y	G	G	A	A	E	L	L	G	L	S	S	P	I	T	K	H	V	L	R	L	Y	T	T	O	V	V	S	G	G	Y	G	G	M	M	S	L	L	L	D	Y	N	F	N	Q	T	R	V	G	V	G	V	M	L	N	D	L	F	F			
<i>P.multocida</i>	Y	N	P	I	S	N	K	K	G	F	L	O	R	S	M	G	W	S	P	I	T	K	H	V	L	R	L	Y	T	T	O	V	V	S	G	G	Y	G	G	M	M	S	L	L	L	D	Y	N	F	N	Q	T	R	V	G	V	G	V	M	L	N	D	L	F	F			
<i>C.jejuni</i>	N	N	L	F	K	F	F	N	N	K	K	G	F	L	O	R	S	M	G	W	S	P	I	T	K	H	V	L	R	L	Y	T	T	O	V	V	S	G	G	Y	G	G	M	M	S	L	L	L	D	Y	N	F	N	Q	T	R	V	G	V	G	V	M	L	N	D	L	F	F
<i>C.coli</i>	Y	Q	Y	W	R	Y	D	R	W	H	G	A	F	F	L	R	L	G	Y	T	P	R	I	N	N	P	F	V	G	I	Y	A	O	W	N	Z	N	G	Y	G	G	M	M	S	L	L	L	D	Y	N	F	N	Q	T	R	V	G	V	G	V	M	L	N	D	L	F	F	
<i>H.pylori_199</i>	Y	Q	Y	W	R	Y	D	R	W	H	G	A	F	F	L	R	L	G	Y	T	P	R	I	N	N	P	F	V	G	I	Y	A	O	W	N</																																	

The *trans* fatty acids have to be reincorporated in phospholipids, by an as yet unknown mechanism. Whereas the involvement of CTI in solvent tolerance is demonstrated [25], the role of OMPLA in this process still needs to be clarified.

2. Structure

2.1. Fold

Structural studies on OMPLA started with the determination of the nucleotide sequence of the *pldA* gene. The rather hydrophilic character and the lack of long hydrophobic amino acid stretches, indicated that the interaction of OMPLA with the membrane differed from integral membrane proteins such as bacteriorhodopsin [7]. Circular dichroism (CD) measurements indicated a high β -strand content [26] and this led Brok et al. [27] to propose a molecular model with a β -barrel architecture. Electron crystallography confirmed the β -barrel character; projection maps showed an oval ring-like density large enough to accommodate 12 β -strands. An elongated domain protrudes from the β -barrel with a size of about 10×25 Å [28]. Overexpression and a protocol for refolding of inclusion bodies [26] paved the way for detailed structural analysis by X-ray crystallography. Crystals suitable for X-ray analysis were grown using detergent solubilised protein and an organic solvent as precipitant [29]. The X-ray structure was published recently [30].

OMPLA has a 12 stranded antiparallel β -barrel composed of a convex and a flat side with approximate dimensions of $20 \times 30 \times 45$ Å³ (Fig. 2A,B) [30]. Amphipathic β -strands traverse the membrane, presenting a cylindrical hydrophobic protein surface. On either side of the hydrophobic cylinder, regions occur which are rich in aromatic residues. Neighbouring β -strands are joined by loops and turns which have a polar character. The enzyme's interior is polar and contains an intricate hydrogen bonding network providing a rigid structure. Two internal cavities exist that contain water molecules, which are capped by hydrophobic residues. Epitope insertion studies [31] identified the orientation of OMPLA in the membrane; the loop region is located at the extracellular

side while the termini and turns are oriented towards the periplasm.

2.2. Active site

First hints for the active site residues were circumstantial, when it was found that *trans*-esterification with methanol resulted in fatty acid methyl esters [3], similar to the lipases from the serine hydrolase family. This suggested that a serine might be a catalytic residue. Later, the finding of an inactive Ser152Phe variant of OMPLA suggested that residue 152 was the active site serine [27]. This hypothesis was rejected, however, when site directed mutagenesis demonstrated some tolerance at this position (Ser152Asn and Ser152Thr) [32]. Definite evidence for a serine as catalytic nucleophile came from inhibition studies with hexadecanesulphonyl fluoride. A stoichiometric amount of hexadecanesulphonyl fluoride led to an irreversible loss of enzyme activity and resulted in sulphonylation of serine 144 [33]. Site directed mutagenesis of Ser144 supported this finding; only the Ser144Cys mutant had some residual activity (approx. 1%). This has been observed more often for serine-cysteine mutations in the serine hydrolase family [32].

The finding of the active site serine led to the belief that OMPLA could be a member of the class of serine hydrolases and could have a classical Ser-His-Asp triad. Inactivation experiments using *p*-bromophenacyl bromide suggested that one out of the five histidines was involved in catalysis [7]. Later, this inhibition proved to be rather non-specific. Nevertheless, site directed mutagenesis unequivocally demonstrated that one histidine, His142, was catalytically essential, with the His142Gly variant displaying a four orders of magnitude lower activity than the wild-type enzyme [34].

The active site residues His142 and Ser144 are located at the exterior of the β -barrel, at the outer leaflet side of the membrane. This location indicates that under normal conditions the substrate and the active site are physically separated, since in *E. coli* phospholipids are exclusively located in the inner leaflet of the outer membrane. The constellation of active site residues resembles that of classical serine hydrolases, however, with an asparagine residue

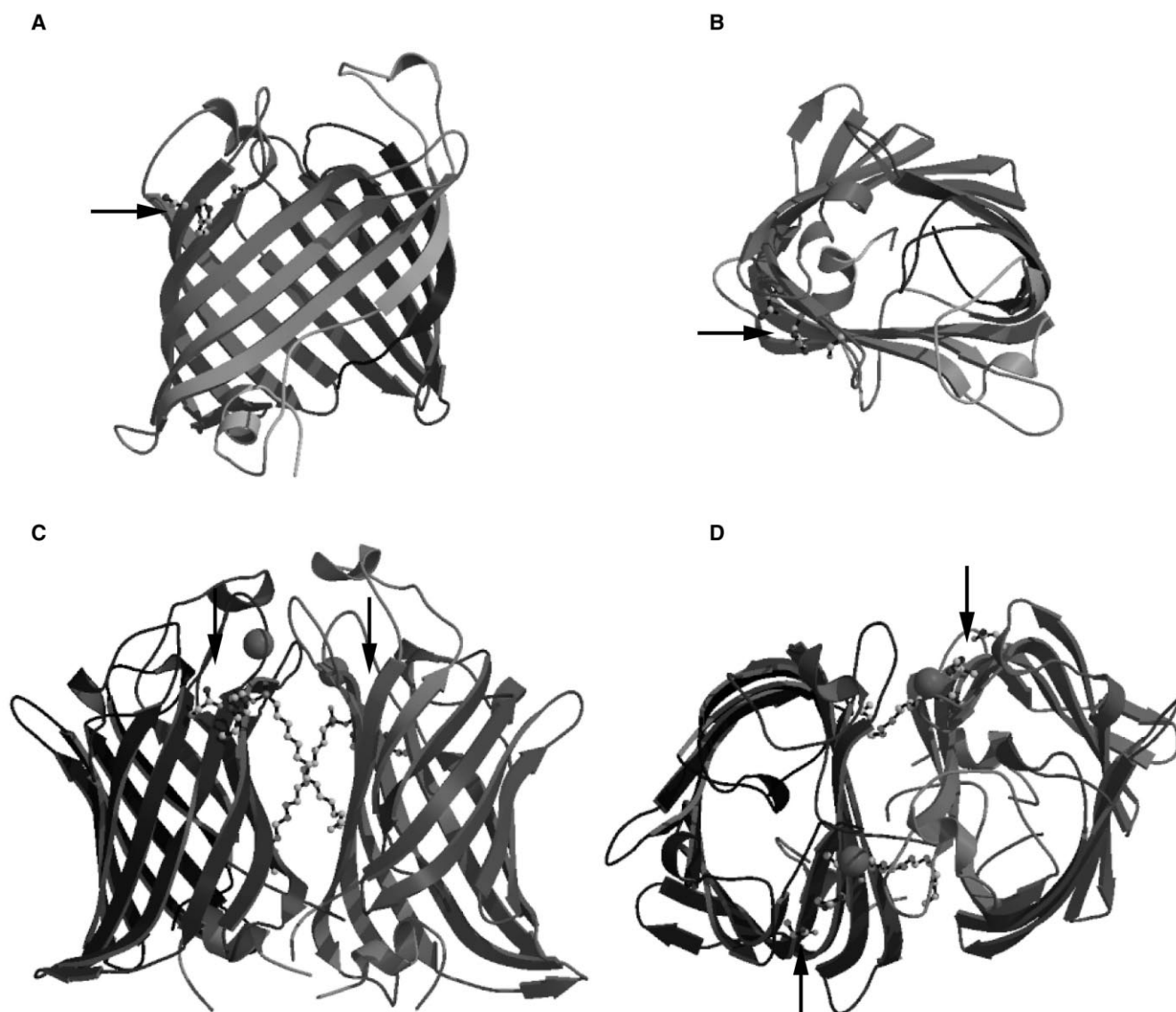


Fig. 2. Orthogonal views of the crystal structures of the OMPLA monomer (A,B) and dimer (C,D). Active site residues and the hexadecanesulphonyl moiety are represented in ball-and-stick, calcium ions are represented by large spheres. The active sites are indicated by an arrow. Molscript [44] and Raster3D [45] were used to prepare this figure.

(Asn156) orienting the histidine residue instead of an aspartate residue in the classical serine hydrolase triad. Recent site directed mutagenesis studies confirmed the importance of Asn156 in catalysis. However, the asparagine residue shows only a modest contribution to catalysis, as an Asn156Ala mutant retained 5% residual activity [46]. Moreover, while Ser144 and His142 are strictly conserved, Asn156 can vary among the different species; an aspartate residue is observed in *Panthoea agglomerans* OMPLA [27] and a glutamine residue is observed in *H.*

pylori OMPLA [35,36]. Furthermore, the Asn156Asp substitution in *E. coli* OMPLA yields an active species [46]. Glutamine and asparagine carboxamides are chemically identical and should impose no catalytic difficulty except for a change in active site geometry. Similar variations have been observed in lipases as well, where both aspartate and glutamate may occur in catalytic triads. Thus, OMPLA belongs to the family of serine hydrolases and demonstrates the suitability of an asparagine as the third residue in the catalytic triad.

3. Activation and regulation

3.1. Dimerisation

3.1.1. Evidence for dimers

Strict regulation of the phospholipase activity is necessary since uncontrolled activity can have lethal consequences for the bacterium [16]. Regulation must occur at the protein level, since OMPLA is constitutively expressed and overproduction causes no phenotypic variations [14,37]. In vitro, the phospholipase activity is modulated in a calcium dependent manner by reversible dimerisation. Inhibition by hexadecanesulphonyl fluoride stabilises the dimeric form of OMPLA [38]. In vivo, chemical cross-linking studies indicate the presence of the monomeric form of OMPLA in the outer membrane [38]. Sonification or induction of bacteriocin release protein activates OMPLA which is a process concurring with dimerisation [39].

Fluorescence resonance energy transfer (FRET) and chemical cross-linking experiments showed that dimerisation is dependent on the presence of calcium and substrate [40]. However, these experiments also supplied evidence for another form of regulation, modulated by the physical state of the outer membrane lipids. In a bilayer environment, dimeric OMPLA displays low activity even in excess of calcium. Perturbation of these bilayers by detergents results in a 70-fold increase in activity.

3.1.2. Dimer structure

OMPLA dimers are formed by the association of two enzyme monomers interacting via the flat barrel side (Fig. 2C,D) [30]. The association mode observed in the crystal structure is supported by cross-linking experiments using H26C single cysteine mutants. Residue 26 is located close to the flat side of the β -barrel OMPLA, and cysteine residues at this position can be effectively cross-linked to produce an active dimer complex [40]. Dimeric and monomeric OMPLA hardly exhibit structural differences, only the N-terminal loop, which precedes the first strand, lacks interpretable density in dimeric OMPLA. Comparison of the X-ray crystal structure and electron microscopy projection maps of monomeric OMPLA indicate a high flexibility of this loop [28] (Snijder et al., manuscript in preparation).

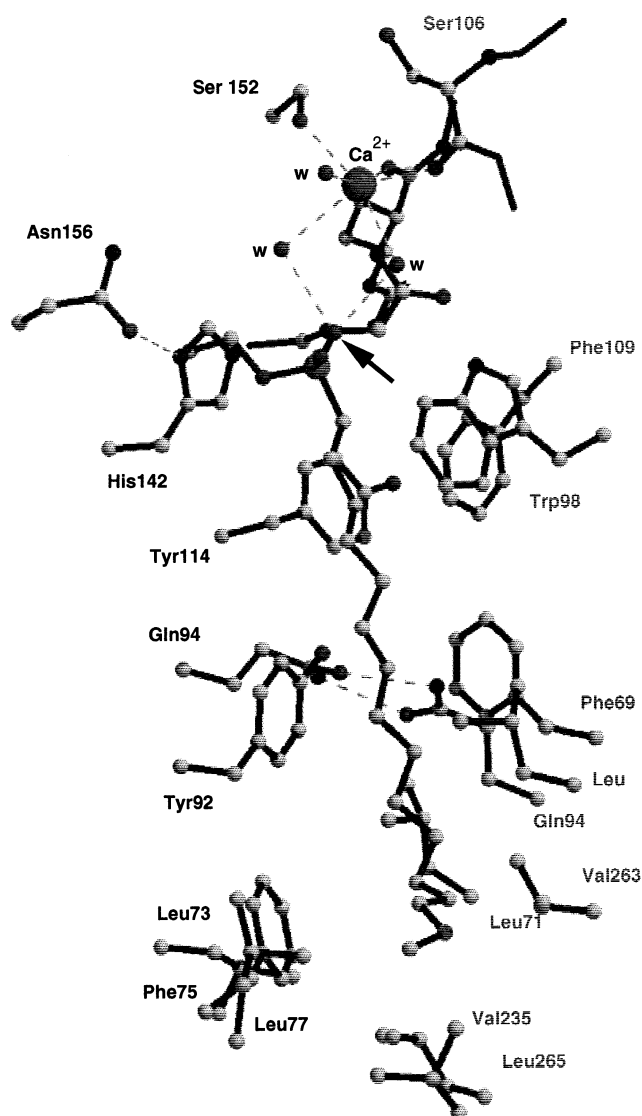


Fig. 3. Close up of the active site of the dimeric complex covalently inhibited with hexadecanesulphonyl. All residues that are involved in substrate binding and catalysis are shown in ball-and-stick, grey and black labels discriminate the two OMPLA molecules in the dimer. The arrow indicates the sulphonyl oxygen that occupies the oxyanion hole. Molscrip [44] and Raster3D [45] were used to prepare this figure.

3.1.3. Formation of substrate binding pockets

Dimerisation creates two extended clefts along the subunit interface which run down from the two active sites. Both clefts are approx. 25 Å long with a sharp bend after 15 Å, and they are lined with hydrophobic and aromatic residues. These clefts bind all 16 carbon atoms of the inhibitor hexadecanesulphonyl fluoride (Fig. 3). Both monomers interact with the

inhibitors and this explains the observed stabilisation of the dimer upon inhibition. The clefts bind only one acyl chain from the substrate. The residues that line these pockets are not strictly conserved, but most mutations are conservative and maintain the hydrophobic character of the pockets. Thus, although a monomer contains a catalytic triad, the monomeric enzyme form lacks catalytic activity due to absence of substrate binding pockets, which are formed only in the dimeric complex [30].

The architecture of the catalytic site and substrate binding clefts allow a broad range of substrates to bind. The active sites are exposed to the solvent and can accommodate a large variety of polar head groups, varying from as small as a glycol unit to a complete Tween 20 head group (polyoxyethylene (20) sorbitan). As a consequence of the one rather non-specific acyl binding site, substrates with either one or two acyl chains of various lengths can be productively bound. Long hydrocarbon chains can extend in the substrate binding pocket and into the hydrophobic membrane core. Short chain length substrates (≤ 12 carbon atoms) are less effectively hydrolysed [8], which is probably related to their lesser efficiency to induce dimerisation as only the carbon atoms at the end of the acyl chain (from C12) have interaction with the neighbouring molecule.

3.1.4. Protein-protein interactions

The dimer interaction surface is almost exclusively confined to the hydrophobic membrane embedded part. Only three main chain hydrogen bonds form the interactions in the polar loops and turns region of OMPLA [30]. In the hydrophobic part, the interface contains a patch of four leucines on both monomers which have a knob-and-hole interaction. Although these are not fully conserved, the hydrophobic character of the interaction is maintained. Additional stabilisation of the dimer complex occurs via aromatic stacking, conserved in all but the *H. pylori* OMPLA. However, the key feature in dimerisation is a double hydrogen bond formed between the Gln94 residue side chain of both monomers, a residue which is embedded in the hydrophobic interior of the dimer. The importance of this polar protein-protein interaction is exemplified by the evolutionary conservation of Gln94 in all *pldA* sequences.

3.2. Calcium dependence

3.2.1. Biochemistry

Calcium is an essential cofactor for activity of OMPLA [6]. It is rather strongly bound, with a dissociation constant of 10–15 μM . Calcium can be replaced by strontium, albeit with a 10-fold higher dissociation constant and a substantially lower activity. Magnesium and barium cannot replace calcium as the catalytic cofactor. They are in fact competitive inhibitors with dissociation constants in the millimolar range [8]. In vitro, calcium is required for dimerisation [38]. Kinetic experiments indicate the influence of the detergent on the calcium affinity. A 70-fold lower affinity (millimolar range) is observed in essays with non-ionic detergents compared to essays with alkylphosphocholine detergents. Various direct binding techniques (gel filtration, fluorescence spectroscopy and isothermal titration calorimetry) characterised calcium binding in detail [41]. Two binding sites are present per monomer, one high affinity calcium binding site ($K_d \approx 36 \mu\text{M}$) and a second binding site with a 10-fold lower affinity ($K_d \approx 358 \mu\text{M}$). As dimerisation occurs at low Ca^{2+} concentration, this suggests that the first calcium ion plays a role in dimer stabilisation. Sulphonylation of OMPLA (by hexadecanesulphonyl fluoride) changes the calcium binding behaviour and two high affinity ($K_d \approx 48 \mu\text{M}$) calcium sites were detected in the inhibited enzyme [41].

3.2.2. Structural evidence

One calcium binding site was identified in the structure of monomeric OMPLA. The calcium ion in this site is located approx. 10 Å away from the active site and is bound between loops L3 and L4 (L3L4 site). Calcium could be substituted for the lanthanide ion Sm^{3+} . Two aspartate side chain carboxylates act as ligands (Asp149 and Asp184). These residues show some evolutionary variation. Aspartate 149 is replaced by arginine in the *Neisseria meningitidis* and *Neisseria gonorrhoeae* OMPLA. In *H. pylori* and *Bordetella pertussis* OMPLA this aspartate is replaced by a glycine, while aspartate 184 is replaced by a serine and a glutamic acid residue respectively. Such variation strongly suggests that enzymes from the different sources will show a different calcium dependence, most likely changing the dimer-

isation behaviour as well as the calcium dependence of activity (Snijder et al., manuscript in preparation).

After dimerisation a second Ca^{2+} binding site is present. It is located in the active site at the dimer interface (catalytic calcium site). The calcium ion is ligated by the side chain of Ser152 and by one main chain carbonyl oxygen atom from each monomer. Water molecules complete the ligation sphere resulting in a binding site with a bipyramidal octahedral geometry. The contribution of both monomers to the binding of calcium correlates with the biochemical evidence that calcium is needed for dimerisation.

The structures supply only relative information on the binding affinities of the two different calcium binding sites. In the dimeric crystal structure, the affinity of the catalytic calcium site is higher than that of the L3L4 binding site, since only the catalytic calcium ion could be modelled with confidence in the experimental electron density maps. The opposite situation occurs in the crystal structure of monomeric OMPLA, where the calcium ion could only be observed in the L3L4 binding site.

Calcium has an essential catalytic role, which is illustrated by the dimeric hexadecanesulphonyl-inhibited structure. This hexadecanesulphonyl inhibitor mimics the negatively charged tetrahedral intermediate of the acylation reaction of serine hydrolases. In classical serine hydrolases, stabilisation of the negative charge during catalysis is supplied by donation of two or three hydrogen bonds by a structural feature, which is called the oxyanion hole. In OMPLA, the sulphonyl oxygen atom which proposedly would develop a negative charge during catalysis, forms hydrogen bonds with the main chain amide from residue glycine 146 and two water molecules. The calcium ion positions these water molecules in the active site and polarises them. Thus, the calcium ion stabilises negatively charged intermediates during catalysis via water molecules.

In the OMPLA Michaelis-Menten complex, the substrate would be oriented with the carbonyl oxygen atom of the ester towards the calcium ion. This orientation causes the carbonyl bond to become polarised and increases the electrophilicity of the carbonyl carbon atom, which will facilitate the nucleophilic attack by serine 144. Thus, the contribution of calcium to catalysis stems (i) from the polarisation of the substrate ester carbonyl group and (ii) from sta-

bilisation of negatively charged reaction intermediate. These roles of the calcium ion suggest that the catalytic importance of the strictly conserved Ser152 is related to its calcium ligating capacity and its contribution to correctly positioning of the calcium ion [30].

3.2.3. Binding model

The various parameters that influence calcium binding and that modulate the affinity of OMPLA for calcium, hamper a full understanding of the complex calcium binding process. Nevertheless, a working model for calcium binding emerges from the combined structural and biochemical data. In the natural environment of OMPLA, calcium is bound to monomeric phospholipase with high affinity. Only after dimerisation, the catalytic calcium site is formed. Active site occupation and dimerisation results in an increased calcium affinity of the active site calcium binding site. Subsequent transfer of calcium to the catalytic site results in the active complex. A second calcium can be now bound to the empty binding site between loops L3 and L4 yielding a dimer complex with two calcium ions per monomer. Although the role of calcium in catalysis seems clear, this is much less so for its role in the regulation of the activity of OMPLA. As calcium ions are abundant in the bacterial outer membrane environment, it is questionable whether the *in vitro* observed calcium dependent dimerisation is of any physiological relevance.

4. Mode of action

All biochemical and structural data lead to a model for the action of OMPLA, which is graphically summarised in Fig. 4. Normally, OMPLA is present in the outer membrane as a monomer with a calcium ion bound at the L3L4 binding site. This form is inactive owing to the absence of substrate binding pockets, lack of oxyanion stabilisation and physical separation of substrate and active site. External or internal triggers (e.g. bacteriocin release protein induction, temperature shock, polymixin B exposure) perturb the outer membrane, introducing phospholipids in the outer leaflet. The presence of the phospholipids in the outer leaflet increases the fluidity of

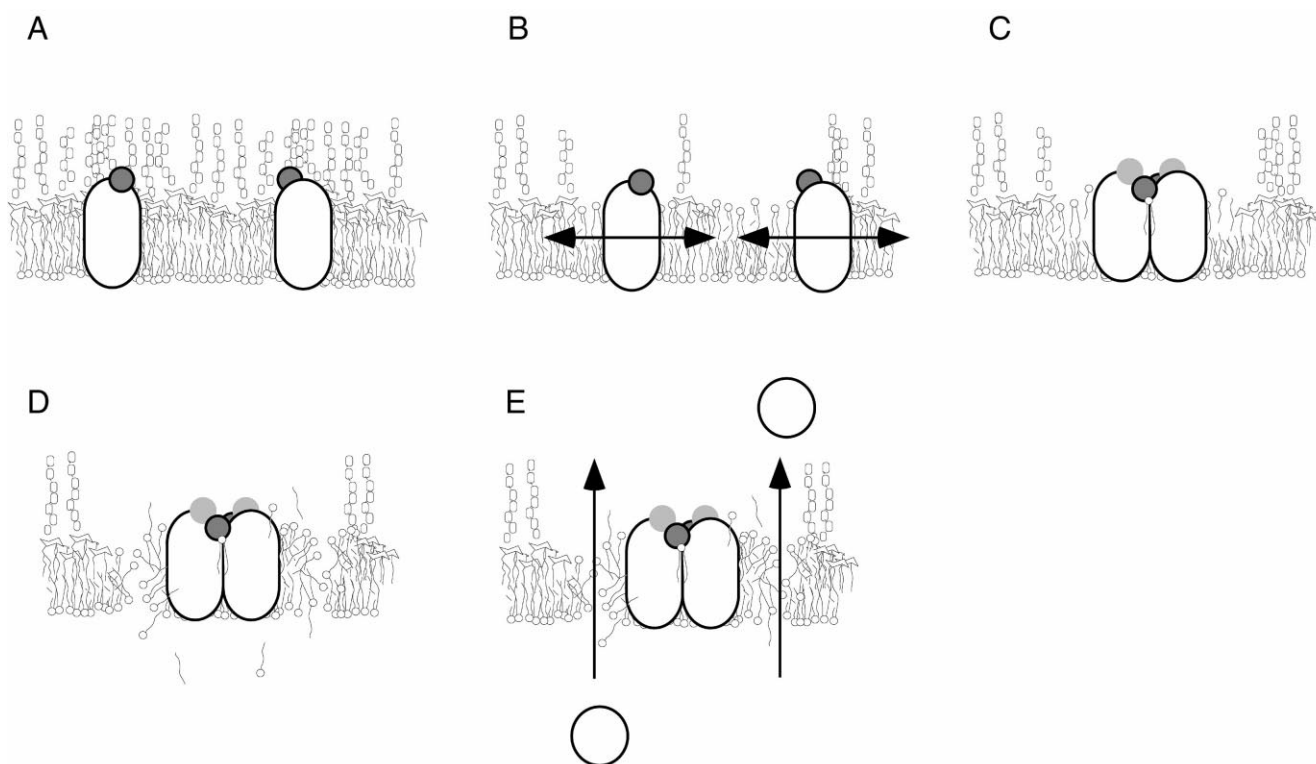


Fig. 4. Model for involvement of OMPLA in bacteriocin release. Under normal conditions OMPLA is in a dormant monomeric form and the outer leaflet of the membrane consists solely of LPS (A). Under the influence of a trigger, for example the bacteriocin release protein, the membrane bilayer is perturbed and phospholipids are presented in the outer leaflet (B). OMPLA dimers are formed, substrate and calcium are bound in the active site (C). Phospholipids are hydrolysed, lysophospholipids and fatty acids perturb the lipid bilayer (D). Eventually the outer membrane becomes permeable and bacteriocins can be semispecifically secreted (E).

the outer membrane, which will facilitate lateral diffusion of its embedded components. Besides that, substrates are presented to the active site of OMPLA, which presumably triggers dimerisation. The calcium ion possibly moves from the L3L4 site to the active site of OMPLA, thus creating an active dimer-substrate-cofactor complex.

The active complex hydrolyses the phospholipid through a mechanism analogous to that of serine hydrolases. Ser144 performs a nucleophilic attack on the carbonyl carbon of the ester, facilitated by polarisation of the ester carbonyl bond by electrostatic interactions with the calcium ion. A negatively charged tetrahedral intermediate is formed which is stabilised by the tetrahedral arrangement of hydrogen bonds and by the remote influence of the calcium ion. The transient intermediate collapses to give the enzyme-acyl intermediate. The lysophospholipid can leave by lateral diffusion in the membrane. Deacylation will occur with a water molecule acting as the

nucleophile. The fatty acid product can leave the active site either by lateral diffusion into the membrane via the opening between the two monomers or alternatively by dissociation of the dimer into monomers.

In addition to the membrane perturbing trigger, release of fatty acids and lysophospholipids destabilises the membrane, possibly resulting in non-bilayer structures. In vitro the OMPLA activity is strongly enhanced by non-bilayer structures, resulting in more rapid generation of fatty acids and lysophospholipids. Hence, the membrane perturbing signal is amplified and the reaction products further permeabilise the membrane. Thus, semispecific excretion of colicins and other effector molecules is facilitated.

5. Conclusion and perspective

The recently solved structures of OMPLA have

yielded a better understanding of membrane protein interactions, phospholipid metabolism, and functioning of this specific phospholipase in particular. It revealed an entirely new fold of phospholipases and sheds light on its catalytic properties. However, our understanding is far from complete. What is the biological relevance of the L3L4 calcium binding site? How can the physical state of the lipidic surrounding modulate enzyme activity? What is the exact biological function of OMPLA? How is OMPLA involved in bacterial pathogenesis? What mechanism arrests enzymatic activity and averts complete bacterial lysis? In the future, continued biochemical and structural research are expected to unravel the remaining secrets and to obtain a full understanding of the molecular mechanism of this intriguing enzyme.

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